Electronic Supplementary Information (ESI)

Microfluidic platform for combinatorial synthesis in picoliter droplets

Ashleigh B. Theberge, Estelle Mayot, Abdeslam El Harrak, Felix Kleinschmidt, Wilhelm T. S. Huck, Andrew D. Griffiths**

Supplementary data

Table S1. LC and MS analysis of 3-component Ugi-type reaction in droplets and bulk

Compound [a]	**LC retention (min)** Droplets (bulk)	**m/z from LC-MS** Droplets (bulk)	**m/z from HRMS** [b] Experimental (theoretical)
1-8	1.59 (1.60)	480.28 (480.24)	480.201* (480.203)
1-9	1.32 (1.32)	475.31 (475.39)	475.242 (475.245)
1-10	1.57 (1.60)	533.31 (533.40)	533.234 (533.251)
2-8	1.34 (–) [c]	455.18 (–)	455.209 (455.208)
2-9	– (–)	– (–)	– (450.250)
2-10	1.31 (1.33)	508.33 (508.38)	508.254 (508.255)
3-8	1.61 (1.60)	486.26 (486.32)	486.172 (486.159)
3-9	1.30 (1.32)	481.26 (481.30)	481.194 (481.202)
3-10	1.56 (1.60)	539.30 (539.37)	539.206 (539.207)
4-8	1.18 (–)	463.33 (–)	– (463.234)
4-9	0.86 (0.96)	458.42 (458.46)	– (458.276)
4-10	1.14 (1.13)	516.36 (516.40)	516.283* (516.282)
5-8	1.36 (1.35)	373.26 (373.27)	373.201* (373.202)
5-9	1.09 (1.06)	368.27 (368.31)	368.243 (368.244)
5-10	1.21 (1.18)	426.33 (426.36)	426.250* (426.250)
6-8	1.54 (1.56)	415.29 (415.35)	415.249* (415.249)
6-9	1.24 (1.27)	410.33 (410.39)	410.291* (410.291)
6-10	1.47 (1.46)	468.34 (468.41)	468.302* (468.297)
7-8	1.80 (1.86)	541.31 (541.36)	541.260* (541.260)
7-9	1.49 (1.52)	536.37 (536.44)	536.302 (536.302)
7-10	1.72 (1.74)	594.37 (594.44)	594.316* (594.307)

[a] Product of the Ugi-type 3-component reaction with the aldehyde and amine indicated in this column and isocyanide **11**. Compound numbers correspond to those in Scheme 1 and Figure 3. [b] When possible, samples from the reaction in bulk were analyzed with high precision where no more than 15 ppm error is expected between observed and theoretical values; these data are indicated with an asterisk (*). For the other samples, no more than 100 ppm deviation is expected between observed and theoretical values. In all cases, the observed values were within the expected error. The masses indicated are for the protonated product, detected in ESI positive ion mode. [c] Dashes (–) indicate that the presence of the desired product was not observed.

Thrombin kinetics in droplets

Bovine thrombin kinetics were measured in individual droplets (~125 pL in volume) using laser-induced fluorescence by taking fluorescence measurements at designated measurement points in a 'delay line' microfluidic device. Delay line microfluidic devices, previously described by Frenz *et al.*, allow for reproducible droplet incubation and periodic spectroscopic measurements.[1] The kinetic plot indicates a linear relationship between droplet residence time and enzymatic activity (Figure S1) as expected based on thrombin kinetics measured in bulk in microtiter plates. This result demonstrates the ability to measure thrombin kinetics in droplets on chip, which could be coupled with the combinatorial synthesis of potential thrombin inhibitors in droplets to produce a combined drug candidate synthesis and screening platform using droplet-based microfluidics.

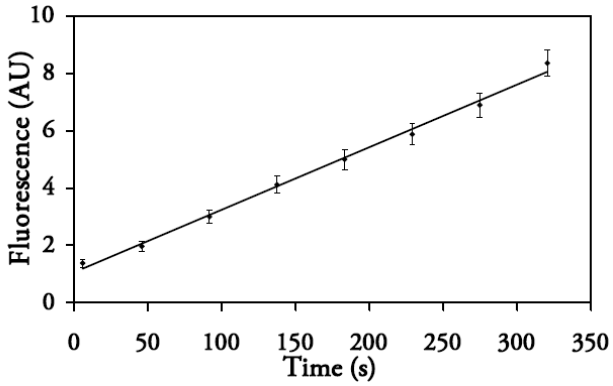

Figure S1. Bovine thrombin kinetics measured in droplets in a delay line microfluidic device. Each data point represents the average of 28,500 droplets; error bars indicate the standard deviation. The results indicate that thrombin is active in droplets and that a delay line style microfluidic device can be used to quantitatively follow the enzyme activity in droplets by detecting the fluorescent product of the enzymatic reaction. The plot indicates a linear relationship between droplet residence time and fluorescent product formation (R^2=0.995).

Experimental details for measurement of thrombin kinetics in droplets

For the measurement of thrombin kinetics in droplets (Figure S1), fluids were dispensed from RDT 1000 Input/Output Vials (RainDance Technologies), with flow rates maintained using custom-built controlled delivery modules (CDMs; IDEX Corp.) and software. The CDM is a miniature, low-power pumping system that provides precise flow measurements from an integrated flow sensor. A proportional-integral-derivative (PID) controller uses the sensor readings to dynamically adjust the flow output, maintaining a precise flow rate (±0.1% over 1 hour). Flow rates were as follows: 50 µL/h (enzyme), 50 µL/h (substrate), 100 µL/h (extra buffer), 150 µL/h (oil). Preparation of enzyme and substrate solutions is described subsequently; 3 wt-% EA-surfactant (RainDance Technologies) in FC-40 was used as the continuous phase (oil). Droplet fluorescence was measured using a custom laser-induced fluorescence setup. The fluorescent product was excited at 488 nm with a 30 mW Sapphire solid-state laser (Coherent, Inc.). A neutral-density filter (ND2) was placed in front of the laser to reduce the laser power. The laser light was focused with a 20x Plan Fluor microscope objective lens with a numerical aperture of 0.45 (Nikon Corp.), which was also used to collect the emitted light. The emitted light was directed to an H9656 photomultiplier (Hamamatsu Photonics KK) using an FF677 dichroic (Semrock) after filtering with a NF01-

488U-25 notch filter and a green FF01-529/28 band-pass filter (Semrock). The microfluidic device was visualized using the 20x objective lens and a 780 nm light-emitting diode (Epoxy-Encased LED780E, Thorlabs, Inc.) as the light source and a Guppy charge-coupled device camera (Allied Vision Technologies GmbH) fitted with a 50 mm macro lens (Stemmer Imaging GmbH).

An alternative procedure was used for PDMS surface modification as follows: the device was treated with 2% 1H,1H,2H,2H perfluorodecyltrichlorosilane in HFE-7100 and then flushed with air. This procedure was repeated once more, and the device was flushed with HFE-7100 and then air and placed in a 65 ºC oven for at least 30 min.

Bovine thrombin (GE Healthcare) was reconstituted and stored according to the supplier's directions: 1x phosphate buffered saline (PBS) (4 ºC) was added to give a final concentration of 0.2 NIH units/µL. Aliquots were stored at -80 ºC prior to use. The substrate, bis-(CBZ-Arg)-rhodamine 110 (Invitrogen), was stored at -20 ºC in aliquots in anhydrous dimethyl sulfoxide (DMSO) at a concentration of 10 mM, as recommended by the supplier. A mixture of 15% (v/v) ethanol and 85% 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 7.5) was used as the assay buffer. The final assay composition was 25 µM substrate, 20 NIH units/mL thrombin, and 0.025 mg/mL bovine serum albumin (BSA, Aldrich) to prevent thrombin deactivation at the droplet-oil interface and adsorption to the tubing and device.

Supplementary videos

The following videos are included as MPEG-4 movie files:

Video S1. Droplets containing amine **10** collected at the outlet of a flow focusing device to be stored and used for subsequent reinjection. The video is played 200 times slower than real time.

Video S2. Reinjection of droplets containing amine **10** after storage off chip for 5 days. The video is played 440 times slower than real time.

References

1 L. Frenz, K. Blank, E. Brouzes, A. D. Griffiths, *Lab Chip*, 2009, **9**, 1344.